*ERJ Express*. Published on December 21, 2018 as doi: 10.1183/13993003.00800-2018

EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Original article

# Systematic and combined endosonographic staging of lung cancer (SCORE Study)

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Please cite this article as: Crombag LMM, Dooms C, Stigt JA, *et al*. Systematic and combined endosonographic staging of lung cancer (SCORE Study). *Eur Respir J* 2018; in press (https://doi.org/10.1183/13993003.00800-2018).

**Systematic and combined endosonographic staging of lung cancer (SCORE Study)**

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**Take-home message:**

In lung cancer patients, a systematic endobronchial (EBUS) combined with an esophageal investigation using the same EBUS-scope (EUS-B) increases the sensitivity for mediastinal nodal staging with 9% compared to a targeted EBUS ('hit-and-run') procedure.

**List of definitions:**

**EBUS-TBNA**: Endobronchial Ultrasound guided-Transbronchial Needle Aspiration. Investigation of mediastinal and hilar lymph nodes with a linear ultrasound probe from the airways with the possibility of nodal sampling under real-time ultrasound control.

**Targeted EBUS-TBNA**: Specific investigation of for malignancy suspected mediastinal and/or hilar lymph nodes based on PET-CT findings (FDG avid or short axis ≥ 10 mm).

**Systematic EBUS-TBNA**: Systematic investigation of all mediastinal and hilar lymph nodes according to the EBUS assessment tool (EBUS-AT) including nodal sampling of suspected nodes (based on PET, CT and/or EBUS) and routinely sampling of station 4R, 4L and 7 (if short axis ≥ 8 mm).

**EBUS-AT**: EBUS assessment tool. Mediastinal and hilar lymph node stations are systematically identified (station 4L, station 7, station 10/11L, station 10/11R, station 4R).

**EUS-B-FNA**: Endoscopic Ultrasound guided-Fine Needle Aspiration using the EBUS scope. A systematic investigation of mediastinal lymph nodes with a linear ultrasound probe from the esophagus according to the EUS-AT with the possibility of nodal sampling under real-time ultrasound control.

**EUS-AT**: EUS assessment tool. Mediastinal lymph node stations are systematically identified (including station 4L, 7, 8 and 9).

**Combined endosonographic staging** (EBUS and EUS-B): Performing a systematic EBUS procedure followed by a systematic EUS-B procedure with sampling of on imaging suspected lymph nodes (PET, CT, EBUS and/or EUS-B) and at least station 4R, 4L and 7 (if short axis ≥ 8 mm). This is a single scope and single operator procedure.

**Clinically relevant staging information** is in a lung cancer nodal staging setting defined as:

- Upstaging to a higher N stage e.g. from N1 to N2, or N0 to N2, or

- Increased number of metastatic involved nodal stations e.g. single level to multilevel N2 disease

**Suspected lymph node** is defined as either:

- Enlarged on CT (short axis ≥ 10 mm), or
- FDG-avid on PET scan, or
- One or more of the following EBUS/EUS features: round shape, short axis ≥ 10 mm, sharp margins on ultrasound or hypo-echogenic texture.

**Sensitivity**: The proportion of patients with N2/N3 metastatic nodal spread that are detected by the diagnostic test.

**NPV**: Negative Predictive Value. The proportion of patients with a negative diagnostic test that do not have N2/N3 metastatic nodal spread.

**Negative Likelihood Ratio:** The probability of a patient who has the disease testing negative divided by the probability of a patient who does not have the disease testing negative.

## Abstract

Introduction:

Guidelines recommend endosonography for mediastinal nodal staging in patients with resectable non-small-cell lung cancer (NSCLC). We hypothesize that a systematic endobronchial (EBUS) evaluation combined with an esophageal investigation using the same EBUS-scope (EUS-B) improves mediastinal nodal staging vs current practice targeted PET-CT-guided EBUS staging alone.

Methods:

Prospective, multicenter, international study (NCT02014324) in consecutive patients with (suspected) resectable NSCLC. After PET-CT, patients underwent systematic EBUS and EUS-B. Node(s) suspicious on CT, PET, EBUS and/or EUS-B imaging and station 4R, 4L and 7 (short axis ≥ 8 mm) were sampled. For patients without N2/N3 disease at endosonography, surgical pathological staging was the reference standard.

Results:

229 patients were included. Prevalence of N2/N3 disease was 103/229 (45%). A PET-CT-guided targeted approach by EBUS identified 75 patients with N2/N3 disease (sensitivity 73% [95% CI 63%-81%], NPV 81% [74%-87%]). Four additional patients with N2/N3 disease were found by systematic EBUS (sensitivity 77% [67%-84%], NPV 84% [76%-89%]) and five more by EUS-B (84 patients total; sensitivity 82% [72%-88%], NPV 87%% [80%-91%]). Additional clinical relevant staging information was obtained in 23/229 patients (10%).

Conclusion:

Systematic EBUS followed by EUS-B increased sensitivity for the detection of N2/N3 disease with 9% compared to PET-CT targeted EBUS alone.

## Introduction

Accurate staging of lung cancer is important because it guides treatment and determines prognosis. In the absence of distant metastases, mediastinal nodal status directs treatment.[1-3] Imaging with CT and FDG-PET has limitations to detect or exclude mediastinal nodal metastases.[4, 5] Therefore, additional testing with tissue confirmation is often indicated, specifically in case of enlarged or FDG avid nodes.[5-7] Guidelines recommend endosonography with fine-needle aspiration as the initial test for mediastinal nodal tissue staging in NSCLC. The latest staging guideline endorsed by the European Respiratory Society (ERS), European Society of Gastrointestinal Endoscopy (ESGE) and European Society of Thoracic Surgeons (ESTS) advises a combination of endobronchial ultrasound with real-time guided transbronchial needle aspiration (EBUS-TBNA) and transesophageal endoscopic ultrasound-guided fine-needle aspiration (EUS-(B)-FNA). A complete assessment of mediastinal and hilar nodal stations, including sampling of at least three different mediastinal nodal stations (4R, 4L, 7) for mediastinal nodal staging is advised.[7] However, strong evidence to recommend a combined and systematic staging approach is lacking. Current EBUS practice commonly involves the so called 'hit and run' strategy where only the (single) on PET-CT suspected node is sampled.[8, 9] Although quick and straightforward, this approach involves the risk of understaging. EBUS and EUS investigations are complementary in their diagnostic range and combined cover the vast majority of mediastinal and hilar nodes.[10] By combining EBUS-TBNA and EUS-FNA, accuracy for mediastinal metastases increases.[11-14] Importantly, an EUS evaluation can be performed with an EBUS-scope (EUS-B), directly following the EBUS procedure, by the same operator, which facilitates a combined approach.[15, 16]

The objective of the current study was to assess the added value of systematic (assessment of all nodal stations, including routine sampling of station 4R, 7, 4L) and combined (endobronchial and esophageal) endosonographic staging using a single EBUS-scope for loco-regional staging (N2/N3 disease), compared to the commonly practiced targeted (PET-CT guided) EBUS staging alone.

## Material and methods

### Study design and subjects

The SCORE study is an investigator initiated, prospective, multicentre, diagnostic accuracy study, performed in both university (n=5) and general hospitals (n=3) in the Netherlands and Belgium. Consecutive patients which (suspected) resectable NSCLC who were medically operable, were eligible for study inclusion if there was an indication for mediastinal nodal tissue verification according to the current guidelines (mediastinal or hilar nodes with short axis ≥ 10 mm, FDG avid mediastinal or hilar nodes, centrally located lung tumor, or primary tumor and lymph nodes that were not FDG-avid).[5-7] Patients were eligible for study inclusion, irrespective of the accessibility of the suspected intrathoracic lymph node to EBUS. So also cases with suspected nodes on station 3A, 5, 6 and 8 were included. Prior diagnostic evaluation included conventional workup (medical history, physical examination, laboratory tests, bronchoscopy), CT scan of the chest and integrated PET-CT. Bronchoscopy was performed either prior to endosonography, or combined in the same setting with the endosonography procedures. Medical operability was assessed by physical examination, blood tests, pulmonary function tests and/or lung perfusion scans. Surgical resectability and medical operability were verified by specialists of a multidisciplinary team. Patients with proven distant metastasis, irresectable disease (as judged by a thoracic surgeon based on the available imaging), small peripheral lung tumors without evidence of enlarged or FDG-positive intrathoracic nodes were not considered for eligibility. Exclusion criteria were mediastinal re-staging after neo-adjuvant treatment, active malignancy with a life expectancy of less than two years, former therapy for lung cancer (chemotherapy, radiotherapy or surgery), technical contraindication for EBUS or EUS (eg, esophagus stenosis), age under 18, pregnancy, or inability to consent. Candidates for study participation were identified at the weekly multidisciplinary lung oncology meeting of the participating centers and provided written informed consent. Prior to endoscopy, the nodal target lesion(s) was/were defined based on PET-CT imaging. Patients underwent a systematic EBUS followed by a EUS-B procedure.[17] In case nodal mediastinal metastases (N2/N3) were found at endosonography, patients were classified as having locally advanced disease (stage III). For patients without pathological evidence of mediastinal metastases at endosonography (N0/N1), surgical verification (mediastinoscopy and/or thoracotomy with nodal dissection) with systematic sampling of mediastinal lymph node stations was performed and considered the reference standard. A multidisciplinary team decided whether to perform surgical staging (mediastinoscopy) or directly surgical lung tumor resection with lymph node dissection. In case no surgical verification was performed (e.g. because pathology revealed SCLC or

benign disease was suspected), at least 6 months of clinical and radiological follow-up including CT scan of the chest was used as an alternative reference standard. The 7th TNM edition of the International Lung Cancer Staging System (IASLC) was used for staging. [18]

This investigator initiated trial was approved by the ethical committees of the eight participating hospitals. This trial was registered as SCORE study (Single scope COmplete staging lung canceR with Endosonography) under number NCT02014324 at clinicaltrials.gov.

**Single scope combined mediastinal staging procedure: systematic EBUS and EUS-B**

EBUS-TBNA and EUS-B-FNA were performed by one operator with an single ultrasound bronchoscope with a linear scanning transducer (Pentax EB-1970UK, Olympus BF-UC180F, Fujifilm EB-530US) with patients under conscious, moderate or deep sedation. All endoscopists were trained in both EBUS and EUS-B and had each performed over 200 procedures.

*STEP 1: Target definition*

Prior to EBUS and EUS-B the nodal target lesion(s) was/were defined based on PET-CT imaging, using the report of the radiologist and the PET-reader. This was used to report the results of targeted EBUS in the hypothetical scenario in which only the pre-defined target lesion(s) was/were sampled.

*STEP 2: Systematic EBUS*

The endoscopist started with full, systematic inspection of the mediastinal and hilar lymph nodes located within reach of EBUS using the EBUS-assessment tool (EBUS-AT) identifying (at least) nodal stations 4L, 7, 10/11L, 10/11R and 4R.[19] After inspection, samples were taken from for malignancy suspected lymph nodes (based on ultrasound and/or PET-CT findings, including the defined nodal target lesion) and routinely sampling of 4R, 4L and 7 was performed (if the node short axis was ≥ 8 mm). Nodal aspirates were taken with 22G or 25G needles and from N3 to N2 to N1 location to avoid upstaging. Suction using a 10 ml syringe during the aspirations was optional. In the absence of rapid on-site examination (ROSE), minimally 2 aspirations of each nodal station were performed.

*STEP 3: Systematic EUS-B*

After the EBUS procedure the EBUS-scope was retracted above the vocal cords and introduced into the esophagus. Inspection of the mediastinal lymph nodes located within reach of EUS was performed in a systematic way using the EUS-assessment tool (EUS-AT) identifying the aorta with celiac trunk, the left adrenal gland (if visible) and lymph node stations 7, 4L and 4R (if visible).[20] Nodal aspirates were taken from suspected lymph nodes and routinely from station 4L and 7 as described above. The nodal stations 4L and 7 were sampled from both the endobronchial and esophageal route if they were suspected on either PET-CT or endosonography.

**Cytology of lymph nodes**

Handling of nodal aspirates was performed according to institutional practice. The outcome of the cytological analysis was presence or absence of malignant cells. The presence of lymphocytes was regarded as a proof for a representative lymph node puncture. The presence of malignant cells was considered to be a true positive result since false positive EUS/EBUS findings are extremely rare.[21, 22] Atypical cells in cytology were classified as benign (no malignancy present).

**End points**

The primary study endpoint was the sensitivity and negative predictive value for mediastinal nodal disease (N2/ N3) of combined endosonographic staging (by EBUS-TBNA and EUS-B-FNA) in comparison to targeted EBUS-TBNA alone.

The secondary endpoints were (1)The sensitivity, negative predictive value and negative likelihood ratio for mediastinal nodal disease (N2/N3) of systematic EBUS-TBNA in comparison to PET-CT directed assessment of the mediastinum (i.e. targeted EBUS); (2) Detection of clinically relevant staging information by combined endosonography (systematic EBUS and EUS-B); (3) Feasibility to detect the left adrenal gland by EUS-B; (4) Serious adverse events of EBUS and EUS-B; (5) Procedure time of EBUS and EUS-B.

**Statistical analysis**

A sample size of 215 was calculated to demonstrate a 7% increase (80 to 87%) in the sensitivity to detect loco-regional disease with combined endosonographic staging (systematic EBUS-TBNA + EUS-B-FNA) compared to targeted EBUS staging alone, assuming a prevalence of locally advanced disease (N2/N3) of 50% and a

dropout rate of 5% with a power of 1-B = 0.80, type 1 error a = 0.05, two-sided testing. We calculated estimates of increase in sensitivity and NPV of targeted EBUS, systematic EBUS and the combined approach. We calculated 95% confidence intervals (CIs) around these proportions using the normal approximation. In case an endosonographic procedure could not be performed (e.g. in case a needle aspiration of a specific lymph node could not be performed safely due to unrest of the patient), the result of that specific procedure was considered to be 'negative' (i.e. no mediastinal nodal disease) in the calculation of accuracy estimates of that specific procedure in order to avoid overestimation of the diagnostic accuracy of the procedure. Patients that did not undergo surgical verification or clinical and radiological follow-up after negative endosonography, were excluded from calculation of sensitivity and NPV (n=4). Statistical analyses were performed using SPSS 24.0 (IBM SPSS Statistics).

## Results

Between May 2013 and October 2014, 280 consecutive patients with (suspected) NSCLC were assessed for eligibility. 229 patients (148 (65%) men, mean age 67 years) were included. Baseline characteristics and study flow of enrolled patients are presented in Table 1 and Figure 1, respectively.

### Final diagnoses

The prevalence of mediastinal nodal metastases was 45% (103/229). The final diagnoses of the 229 patients were NSCLC (n=188; 82%) SCLC and Large-Cell Neuroendocrine Carcinoma (LCNEC) (n=17; 7%); benign e.g. granulomatous disease, pulmonary infection (n=14; 6%); suspected lung cancer (n=8; 4%) and other (n= 2; 1%) (Table 1).

### Targeted EBUS ('Hit-and-Run' Strategy)

227 patients underwent a targeted EBUS with sampling of lymph node(s) suspicious on PET-CT detecting mediastinal nodal metastases in 75 patients. In 2 patients it was not possible to introduce the EBUS scope into the trachea. A median of 1 hilar and/or mediastinal target lesion(s) was defined prior to endosonography (range 0-8) and a median of 1 target lesion was sampled (range 0-4).

**Added value of systematic EBUS**

215 patients underwent a systematic EBUS procedure with systematic inspection of the mediastinal and hilar lymph nodes, revealing four additional patients with mediastinal disease compared to PET-CT guided targeted EBUS (three on station 7, and one on both station 7 and 4R). In 14 patients, systematic EBUS (including routinely sampling) could not be performed, due to cough or anxiety of the patient. Systematic EBUS revealed clinically relevant staging information in four more patients due to detection of multi-level N2 disease (instead of single level N2 disease) in three and upstaging to N3 disease in one patient (Figure 2 and Supplement 1). A median of two different mediastinal nodal stations (range, 0-4 ) were sampled. A median of 3.0 (range 1-9) lymph node passes per nodal station were taken. Median EBUS procedure time was 20 minutes (range 0-68).

**Added value of systematic EUS-B (following systematic EBUS)**

220 patients underwent a systematic EUS-B procedure after the endobronchial procedure revealing pathological proof of mediastinal metastases in five patients not found by systematic EBUS. These were located in stations 4L (n=2), subcarinal station 7 (n=2) and station 8 (n=2) (in one patient additional nodal metastases were found in 2 different nodal stations). In nine patients no systematic esophageal procedure was performed either because the endoscopist was unable to introduce the EBUS scope into the esophagus, or the EUS-B procedure was aborted due to unrest of the patient. EUS-B revealed also clinically relevant staging information in 11 more patients due to detection of multi-level N2/N3 disease (instead of single level N2/N3 disease) and upstaging to N3 disease (Figure 2 and Supplement 2). At EUS-B a median of one different mediastinal nodal station (range, 0-3) was sampled. A median of 2.0 (range 1-6) lymph node passes per nodal station were taken. Median procedure time was 10 minutes (range 0-45). A mean of 1.4 needle was used (range 0-3) for the combined EBUS and EUS-B procedure. ROSE was available in one third of patients.

**Reference standard and false negative endosonographic findings**

In 84 patients EBUS and/or EUS-B revealed pathological proof of N2 disease (true positive). In six other patients the diagnosis N2/N3 (all in station 5/6) was made based on the combination of PET-CT and endosonography characteristics (round shape, enlarged, sharp margins and hypo-echogenic). Due to position of

these lymph node stations, adequate and safe sampling was not possible due to interposition of vascular structures. For calculation of sensitivity and NPV the results of these six patients were considered false negative in order to avoid overestimation of the accuracy of the endosonography procedures.

Of the 139 patients in whom endosonography revealed no mediastinal metastases (N0/N1), 103 (74%) underwent surgical-pathological verification and 32 (23%) clinical and radiological follow-up. In four patients (3%) a reference standard for mediastinal metastasis was missing (due to death in three) (Figure 1). Surgical nodal verification was performed by mediastinoscopy, VATS or thoracotomy with lymph node dissection. At surgical verification, a median of four different hilar and mediastinal nodal stations (range, 0-8) were sampled revealing nodal metastases in 11 patients. One patient underwent surgical staging after induction chemotherapy, revealing no mediastinal metastases, but after induction therapy lymph node decreased in size and therefore endosonography was considered to be false negative. In one patient radiological follow-up of mediastinal lymph nodes showed growth of one mediastinal lymph node suspicious for nodal metastases after chemoradiotherapy. The endosonography result of this patient was assumed to be false negative. The median duration of radiological and clinical follow-up was eight months (range 3-24 months). At preoperative staging, combined EBUS and EUS-B results were false negative for N2/N3 disease in 13 patients (13%). In 5/13 patients (38%) these nodes were located in stations 5/6/3A, beyond reach of endosonography and in three cases it only concerned micro metastases (Supplement 3).

**Outcomes**

The sensitivity for detecting mediastinal nodal metastases by PET-CT guided targeted EBUS staging alone was 73% (75/103; 95%CI, 63%-81%), for systematic EBUS 77% (79/103; 95%CI, 67%-84%%), and for combined endosonography (systematic EBUS + EUS-B) 82% (84/103; 95%CI, 72%-88%). Negative predictive value of targeted EBUS, systematic EBUS and combined endosonography (systematic EBUS + EUS-B) were 81% (122/150; 95%CI, 74%-87%), 84% (122/146; 95%CI, 76-89%) and 87% (122/141; 95%CI, 80%-91%), respectively. Negative likelihood ratio of targeted EBUS, systematic EBUS and combined endosonography (systematic EBUS + EUS-B) were 0.27 (95%CI 0.20-0.37), 0.23 (95%CI 0.16-0.33) and 0.18 (95%CI 0.12-0.28), respectively. (Table 2).

Systematic EBUS revealed clinical relevant staging information in eight patients compared to targeted EBUS. In 16 patients EUS-B added clinically relevant staging information to the systematic EBUS procedure (Figure 2).

One patient had an added value for both systematic EBUS and EUS-B (Supplement 1 and 2). The number of patients needed to undergo a systematic EBUS followed by a EUS-B procedure to detect clinically relevant mediastinal nodal staging information was ten, compared to the hit-and-run targeted EBUS procedure.

In 94/151 (62%) patients in whom it was attempted to visualize the left adrenal gland, it was feasible to detect the left adrenal gland by EUS-B.

No serious adverse events related to the endosonography procedures were reported. One patient had a fatal intracranial bleeding 48 hours after endosonography. This was judged as unlikely to be related to the endosonography procedure.

## Discussion

A systematic EBUS procedure followed by EUS-B increases the sensitivity for the detection of mediastinal nodal metastases in lung cancer patients with 9% compared to a PET-CT guided targeted EBUS approach. Furthermore, clinically relevant staging information is obtained in one out of ten patients. EUS-B prolongs the endosonography procedure with ten more minutes and the benefit in staging was not associated with complications.

We showed that a targeted EBUS procedure based on PET-CT findings, has a sensitivity for detection of mediastinal nodal disease (N2/N3) of 73%. This is comparable with a large meta-analysis where a mean sensitivity 72% (95%CI 58%–82%) for EBUS was reported.[23] By performing a systematic EBUS, sensitivity increased with 4% in comparison to a targeted EBUS approach. To the best of our knowledge, systematic and targeted mediastinal staging with EBUS-TBNA have not been directly compared previously in a prospective study. A retrospective study in 93 patients reported on systematic nodal sampling in EBUS and concluded that routinely sampling more than two mediastinal stations may improve staging.[24] A recent single center retrospective study reported a 13% increase in important clinical information by systematic EBUS sampling compared to targeted EBUS.[25] We also found that systematic evaluation including routinely sampling of (at least) station 4R, 7 and 4L, if short axis ≥ 8 mm, increases sensitivity for nodal staging compared to PET-CT guided targeted EBUS alone. By introducing the EBUS scope into the esophagus following a systematic EBUS procedure, sensitivity for mediastinal metastases increases with another 5%. A recent systematic review showed

that the addition of EUS(-B) to EBUS leads to 12% gain in the detection of mediastinal nodal metastases.[23] A possible explanation for the lower difference found in the SCORE study is the fact that a systematic EBUS with routinely sampling of mediastinal lymph nodes was performed prior to EUS-B. In addition to access to the lower mediastinum, the esophageal route has several advantages over the endobronchial approach. Nodal sampling is easier due to the absence cartilage rings and cough. Our findings are comparable with the outcomes of the ASTER trial, were both EBUS and conventional EUS were used for mediastinal nodal staging.[26] They reported a sensitivity of 85% for mediastinal nodal staging, similar to our 82%.

Data from the SCORE study also showed that a systematic and combined approach reveals clinically relevant staging information in one out of every ten patients compared to a targeted EBUS approach. This seems relevant because the number of metastatic lymph nodes appears to be a better prognostic determinant than the currently used anatomical location based N classification (N0, N1, N2 or N3) in lung cancer patients.[27, 28] A more accurate assessment of nodal status by a systematic EBUS combined with EUS-B seems relevant for delineation for radiation therapy which is normally done by PET–CT-based selective lymph node irradiation.[29] The current study was adequately powered and was performed in a large and well-defined study population; target lesion(s) based on PET-CT imaging was (were) determined prior to endoscopy and endosonography procedures were performed in a structured and well documented three-step approach. Patients were included in both general and university hospitals. To avoid the risk of overestimation of the accuracy of the endosonography procedures, a conservative analysis was chosen where only patients with a pathologically proven nodal metastasis were considered true positive. In clinical practice, diagnosis of N2/N3 disease is sometimes made based on the combination of PET-CT and endosonography characteristics only, when a nodal aspiration cannot be performed safely (e.g. station 5/6). In this study, EBUS and/or EUS-B detected N2/N3 disease in 90 patients. In 84 patients this was based on tissue proof of nodal metastases. In the other six patients, malignant involvement was made based on the combination of PET-CT and endosonography characteristics only without tissue proof . For accuracy analysis the latter six cases were considered false negative. If these specific six cases were considered true positive, the sensitivity of targeted EBUS vs systematic EBUS vs combined EBUS and EUS-B would be 79% vs 83% vs 87%, respectively. A limitation of our study was that 36/139 lung cancer patients (26%) without evidence of mediastinal nodal metastases after endosonography did not undergo surgical nodal verification. This potentially overestimates the sensitivity and NPV of endosonographic procedures. However, clinical and radiological follow-up was available in almost all patients. Using a single EBUS scope for a combined

endosonographic staging procedure instead of the use of both an EBUS and a separate conventional EUS scope is most likely not a limiting factor, since it has been described previously that both staging approaches are equally effective and safe for mediastinal staging.[23, 30]

In conclusion, among patients with (suspected) NSCLC, a systematic staging strategy combining EBUS and EUS-B resulted in increased sensitivity for mediastinal nodal metastases compared with targeted EBUS alone. Moreover this resulted in meaningful clinical staging information in 10% of the included patients. This single EBUS scope and operator staging strategy might qualify as the optimal way to assess locally advanced nodal disease in lung cancer patients. Future studies should confirm current outcomes and investigate the impact on patient management and outcomes.


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**Table 1. Clinical characteristics of patients in study (n=229)**

| | |
|---|---|
| Age, mean (SD), y | 67 (8,9) |
| Sex, No. (%) | |
| Male | 148 (65) |
| Female | 81 (35) |
| Diagnostic workup prior to endoscopy | |
| FDG-PET-CT, No (%) | 211 (92) |
| Bronchoscopy, No (%) | 135 (59) |
| Tumor stage PET/CT, No. (%) | |
| cT1 | 73 (32) |
| cT2 | 99 (43) |
| cT3 | 50 (22) |
| cT4 | 7 (3) |
| Nodal status PET/CT, No. (%) | |
| cN0 | 35 (15) |
| cN1 | 37 (16) |
| cN2 | 129 (57) |
| cN3 | 28 (12) |
| Final histopathology data, No. (%) | |
| NSCLC (epithelial tumors) | |
| Squamous cell carcinoma | 88 (38) |
| Adenocarcinoma | 77 (34) |
| NSCLC- NOS | 21 (9) |
| Adenosquamous carcinoma | 1 (0.5) |
| Other: Sarcomatoid carcinoma | 1 (0.5) |
| Neuroendocrine tumors | |
| Small cell carcinoma | 11 (5) |
| LCNEC | 6 (3) |
| Suspected lung cancer | 8 (3) |
| Benign lesion | 14 (6) |
| Other * | 2 (1) |

Legend Table 1.

The 7th TNM edition of the International Lung Cancer Staging System (IASLC) was used for staging.

*Lymphoma and metastasis of extrathoracic tumor. LCNEC: Large cell neuroendocrine carcinoma.

Suspected lung cancer: Suspicion of lung cancer based on clinical presentation in combination with imaging characteristics (PET-CT) in the absence of tissue proof of malignancy.

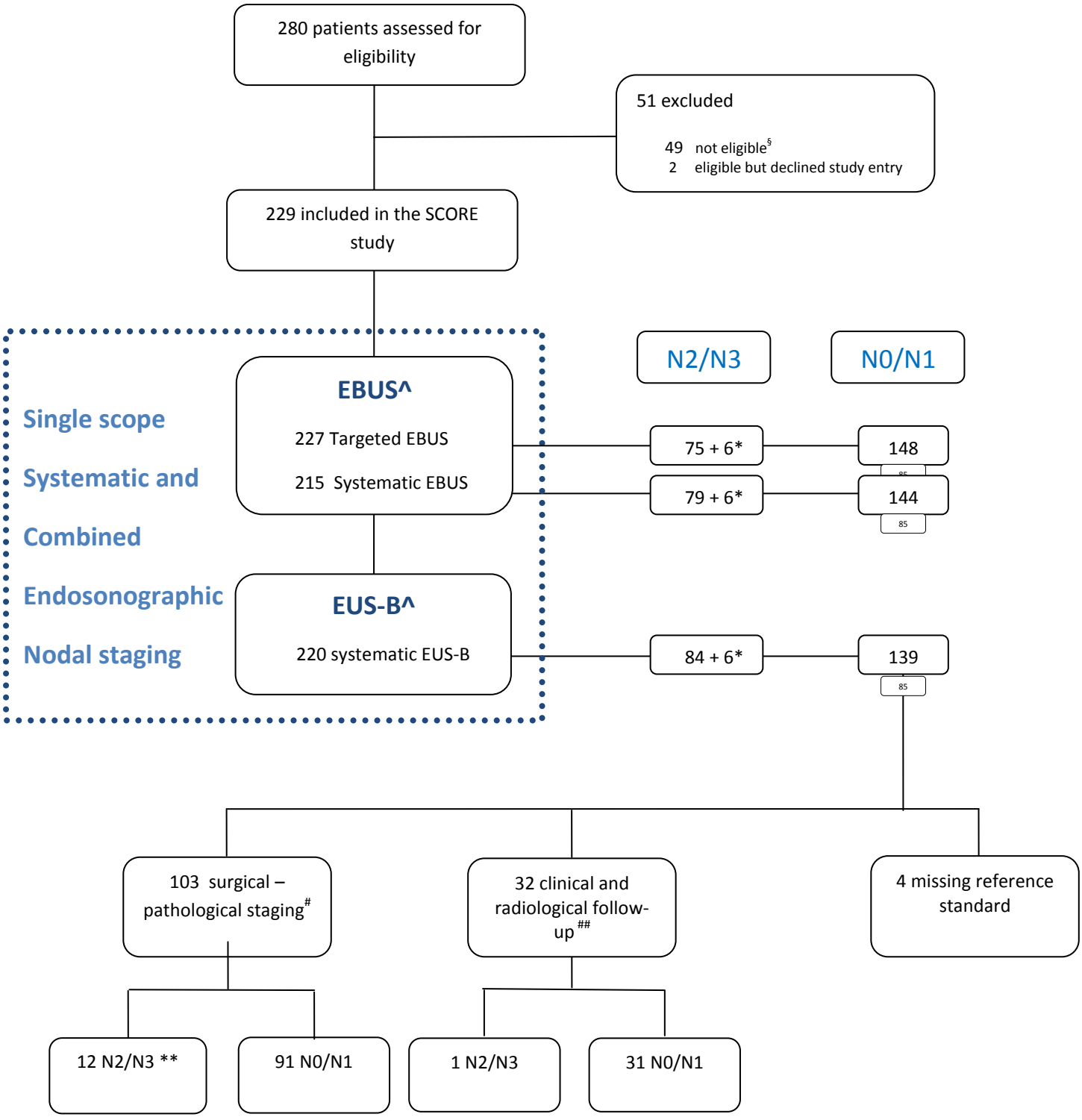
Table 2. Diagnostic performance of endosonography (n=225)

| | **No./Total No. (%) [95% Confidence Interval]** | | |
|---|---|---|---|
| **Nodal invasion, N2/N3 disease** | **Targeted EBUS** | **Systematic EBUS** | **Combined EBUS + EUS-B** |
| Sensitivity | 75/103 (73)<br>[63-81] | 79/103 (77)<br>[67-84] | 84/103 (82)<br>[72-88] |
| Negative Predictive Value | 122/150 (81)<br>[74-87] | 122/146 (84)<br>[76-89] | 122/141 (87)<br>[80-91] |
| Negative Likelihood Ratio | 0.27<br>[0.20-0.37] | 0.23<br>[0.16-0.33] | 0.18<br>[0.12-0.28] |

Legend table 2

Combined EBUS and / or EUS-B detected N2/N3 disease in 90 patients. In six patients these diagnosis was made based on PET-CT and endosonography characteristics only (round shape, enlarged, sharp margins and hypoechogenic). In these six patients pathological proof of N2 disease (all on station 5 / 6) was not obtained by EBUS and/or EUS-B due to interposition of vascular structures. For calculation of sensitivity, Negative Predictive Value and Negative Likelihood Ratio the results of these six patients were considered false negative. If these specific 6 patients were considered true positive the sensitivity of targeted EBUS vs systematic EBUS vs combined EBUS and EUS-B would be 79% vs 83% vs 87% and 85% vs 87% vs 90% for NPV, respectively. Patients that did not undergo surgical verification or clinical and radiological follow-up after negative endosonography, were excluded from calculation of sensitivity and NPV (n=4).

Figure 1. Flow diagram of enrollment and intervention



Legend figure 1:

Flow diagram of enrollment of patients with (suspected) lung cancer and an indication for mediastinal nodal tissue staging who underwent EBUS (endobronchial ultrasound) and EUS-B (endoscopic ultrasound using the EBUS scope).

§ Of 49 patients ineligible for study participation, 32 were deemed as medically inoperable, 8 had previous therapy for lung cancer and 9 patients had an indication for induction therapy.

^ In the 229 included patients, the diagnostic procedures were performed in a fixed sequence: first EBUS and then EUS-B. In 2 out of 229 (1%) patients, the endoscopist was not able to introduce the EBUS scope into the trachea. All remaining 227 (99%) patients underwent targeted EBUS and 215 (94%) of them a systematic EBUS procedure as well.

In 14 (6%) patients a systematic EBUS was not possible (due to cough and/or unrest). Following EBUS, systematic EUS-B was performed in 220/229 (96%) patients (including the two patients in whom the introduction of the EBUS scope into the trachea failed). In 9 (4%) patients no systematic EUS-B was performed due to inability to introduce the EBUS scope into the esophagus after the EBUS procedure or unrest of the patient.

*In 84 patients there was pathological proof of N2 disease by EBUS and/or EUS-B; in six other patients the diagnosis N2/N3 (all in station 5 / 6) was made based on PET-CT and endosonography characteristics (round shape, enlarged, sharp margins and hypoechogenic), since the suspected mediastinal lymph node could only be reached by a transvascular approach (station 5 and 6); For calculation of sensitivity and NPV the results of these six patients were considered false negative.

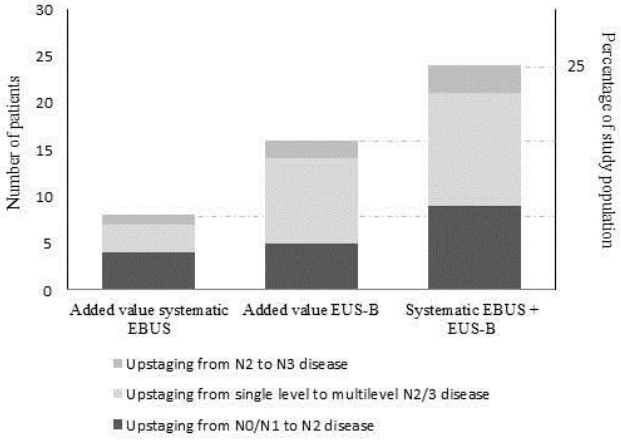
# Delayed surgery in 3 patients after induction therapy.

## The reasons the treating physician or multidisciplinary team refrained from surgery with lymph node dissection were: nonmalignant disease (n=7, diagnosis based on wedge resection, punctures, cultures and/or significant reduction on follow-up imaging); tumor board decision (medically inoperability n=8, irresectable tumor n=2, radiation +/- chemotherapy n=10 e.g. for SCLC); patients refusal (n=5); deceased before surgery

(CVA) n=1; appeared to have pleuritis carcinomatosa at surgery, no lymph node dissection performed n=1; peroperative T4, no lymph node dissection performed n=1; pulmonary metastasis of extrathoracic tumor n=1.

** Surgical verification revealed mediastinal nodal metastases in 11 patients. One patient underwent surgical staging after induction chemotherapy, revealing no mediastinal metastases, but after induction therapy lymph node decreased in size and therefore endosonography was considered to be false negative. So in total in 12 patients endosonography results were false negative.

Figure 2. Additional clinical staging information of systematic and combined endosonography compared to targeted EBUS (n=229)



Supplement 1

Added value of systematic EBUS compared to targeted (PET-CT guided) EBUS for mediastinal nodal staging.

| **Sex and Age (y)** | **Tumour location** | **cTNM (imaging based)** | **Nodal target based PET-CT imaging** | **Final diagnosis** | **Targeted EBUS**<br><br>**sampled LN station(s)** | **Systematic EBUS**<br><br>**sampled LN station(s)** | **Additional nodal metastasis detected by systematic evaluation** | **Change of nodal status**<br><br>**Targeted vs Systematic approach** | **Remarks** |
|---|---|---|---|---|---|---|---|---|---|
| F, 66y | Central, RLL | cT2aN0M0 | None | Adenocarc | NA | 7 | 7 | N0 → N2 | |
| M, 39y | Peripheral, RUL | cT3N1M0 | 11R | Adenocarc | 11R | 7, 4R, 11R | 4R, 7 | N0→ N2 | Sample of station 11R was non-diagnostic |
| M, 68y | Peripheral, RUL | cT1bN1M0 | 10R | Adenocarc | 10R | 7, 10R | 7 | N1 →N2 | |
| M, 74y | Central, ML | cT1aN1M0 | 11R | SCLC | 11R | 11L, 7, 4R, 11R | 7 | N1 → N2 | PET-CT has been revised |
| F, 53y | Peripheral, RUL | cT1aN2M0 | 4R | Adenocarc | 4R | 4L, 7, 4R, | 7 | N2sl → N2ml | |
| F, 63y | Peripheral, RUL | cT1aN2M0 | 4R | Adenocarc | 4R | 7, 4R | 7 | N2sl → N2ml | |
| M, 63y | Peripheral, ML | cT1bN2M0 | 11R, 7 | SCLC | 7 | 4L, 7, 4R | 4R | N2sl → N2ml | Also added value EUS-B on station 8R |
| M, 76y | Peripheral, RLL | cT2bN2M0 | 11R, 4R, 7 | SqCC | 4R, 7, 11R | 4L, 7, 4R, 11R | 4L | N2→N3 | |

Legend Supplement 1:

This supplement contains additional information on the patients in whom performing a systematic EBUS procedure resulted in either upstaging of nodal status from N0/1 to N2/3 or revealed otherwise clinical relevant nodal staging information by upstaging the patient from single level N2 to multilevel N2 or N3 disease compared to a imaging guided targeted EBUS based on CT-PET findings (Hit-and-run approach).

Remark:

Atypical cells in cytology are not classified as malignant.

Abbreviations:

LLL: Left Lower Lobe; LUL: Left Upper Lobe; RLL: Right Lower Lobe; ML: Middle Lobe; RLL: Right Lower Lobe; Adenocarc: Adenocarcinoma; SqCC: Squamous Cell Carcinoma; SCLC: Small Cell Lung Cancer; NA: not applicable; SL: single level; ML: Multilevel;

Supplement 2.

Added value of EUS-B-FNA compared to systematic EBUS-TBNA for mediastinal nodal staging

| **Sex and Age (y)** | **Tumour location** | **cTNM based on imaging** | **Suspected LN station (s) on imaging** | **Final diagnosis** | **Systematic EBUS-TBNA**<br><br>**sampled LN station(s)** | **Systematic EUS-B-FNA**<br><br>**sampled LN station(s)** | **Change of nodal status**<br><br>**Systematic EBUS vs EUS-B** | **Added value of EUS-B (based on station)** | **Remarks** |
|---|---|---|---|---|---|---|---|---|---|
| F, 50y | Central, ML | cT2aN0M0 | None | NSCLC-NOS | 4R, 7, 11R | 7 | N0→ N2 | 7 | Cytology result EBUS on station 7 was benign LN |
| M, 69y | Central, LLL | cT1bN2M0 | 8L | SqCC | 7, 4L | 4L, 7, 8L | N0 → N2 | 7, 8 | Station 8L not found by EBUS; cytology results EBUS on station 4L and 7 were benign LN |
| F, 58y | Peripheral, LLL | cT2aN2M0 | 11L, 4L | Adenocarc | 7, 11L | 4L, 7 | N0 → N2 | 4L | Station 4L was not visible from airways |
| F, 52y | Peripheral, LUL | cT1bN2M0 | 10L, 11L, 4L, 5 | Adenocarc | NA | 4L | N0→N2 | 4L | Suboptimal EBUS due to unrest patient |
| M, 67 | Central, LLL | cT3N0M0 | None | LCNEC | 4L, 7 | 4L, 7,8 | N0 → N2 | 8 | |
| M, 59y | Peripheral, RLL | cT1aN2M0 | 7 | Adenocarc | 4L, 4R, 7 | 4L, 7 | N2sl → N2ml | 4L | Station 4L only at EUS-B malignant |
| M, 72y | Central, LLL | cT2bN2M0 | 11L, 4L, 7 | Adenocarc | 4L, 7 | 4L, 7 | N2sl → N2ml | 4L | Station 4L only at EUS-B malignant |
| M, 63y | Peripheral, ML | cT1bN2M0 | 11R, 7 | SCLC | 4L,4R,7 | 4L, 7, 8 | N2sl → N2ml | 8 | Also added value systematic EBUS |
| M, 51y | Central, ML | cT2aN2M0 | 11R, 4R, 7 | Adenocarc | 4R | 7 | N2sl → N2ml | 7 | Intolerable cough, EBUS aborted / suboptimal EBUS due to cough patient |
| M, 77 | Peripheral, RLL | cT2bN2M0 | 11R,7,8 | SqCC | 7 | 7, 8 | N2sl → N2ml | 8 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| M, 61y | Peripheral, RUL | cT2aN2M0 | 11R, 4R,7 | Adenocarc | 7, 4R, 11R | 4L, 7 | N2ml → N3 | 4L | 4L not suspicious at EBUS (nor EUS-B) |
| M, 64y | Peripheral, RUL | cT3N2M0 | 11R, 4R, 7 | Large cell carcinoma | 4R | 7, 4L | N2sl → N3 | 4L, 7 | Intolerance for EBUS, therefore 4L and 7 sampled from the esophagus |
| M, 59y | Central, LLL | cT2aN2M0 | 10L, 4L | Adenocarc | 4L, 10L | 4L, 7,8 | N2sl → N2ml | 8 | |
| F, 52y | Peripheral RUL | cT1aN3M0 | 11R, 2R, 4R, 2L, 4L | Adenocarc | 11L, 2L, 4L, 4R, 2R, 11R | 2L, 4L, 7 | N3sl → N3ml | 7 | Revision of cytology has been performed |
| M, 69y | Central, RUL | cT3N3M0 | 10R, 4R, 7, 2L, 4L | LCNEC | 2L | 4L, 7 | N3sl→ N3ml | 4L, 7 | EBUS procedure was aborted due to desaturation |
| M, 58 | Central, LLL | cT1bN3M0 | 2L, 4L, 5, 6, 7, 11L | NSCLC-NOS | 7, 11L | 2L, 4L, 7 | N2sl → N2ml | 2L, 4L | 2L and 4L at EBUS unsuspicious, distorted trachea. |

Legend Supplement 2:

Supplement 3.

False negative results of complete endosonographic staging (systematic EBUS + systematic EUS-B) for N2/N3 disease in lung cancer patients.

| **Sex, age (y)** | **Tumor location** | **Final histopathology** | **cTNM based on imaging** | **FALSE NEGATIVE LN station** | **LN suspected based on PET-CT** | **Sampled LN station and number of needle passes at endosonography** | **LN within reach of endosonography** | **Micrometastases (< 2 mm); Intranodal; Extranodal**<br><br>**Remarks** |
|---|---|---|---|---|---|---|---|---|
| M, 56y | Peripheral, RLL | SqCC | cT3N1M0 | 4R and 7 | no | EBUS: 2x TBNA 4R; 2x TBNA 7<br><br>EUS-B: no FNA 4R; 2x FNA 7 | yes | Micrometastases |
| M, 68y | Centrally, LLL | Adenocarcinoma | cT2aN2M0 | 4L | yes | EBUS: 2x TBNA 4L<br><br>EUS-B: 4x FNA 4L | yes | Micrometastases |
| M, 58y | Centrally, LUL | SqCC | cT2bN2M0 (2L and 4L) | 6 | no | ÈBUS: No sampling FN node<br><br>EUS-B: No sampling FN node | no | Intranodal metastases |
| M, 64y | Peripheral, RUL | Adenocarcinoma | cT2aN2M0 | 2R | no | ÈBUS: No sampling FN node<br><br>EUS-B: No sampling FN node | yes | Intranodal metastases |
| M, 78y | Peripheral, LLL | SqCC | cT2aN2M0 | 4L | no | ÈBUS: No sampling FN node<br><br>EUS-B: No sampling FN node | yes | Micrometastases |
| M, 57y | Centrally, LUL | Adenocarcinoma | cT2bN0M0 | 4L | no | ÈBUS: No sampling FN node<br><br>EUS-B: 1x FNA 4L | yes | Intranodal metastases |
| M, 64y | Peripheral, LLL | Adenocarcinoma | cT1aN1M0 | 8 | no | ÈBUS: No sampling FN node<br><br>EUS-B: No sampling FN node | yes | Unknown<br><br>MS also FN result, LND revealed nodal metastasis |
| F, 69y | Peripheral, RLL | Large cell carcinoma | cT3N1M0 | 7 | no | EBUS: 3x TBNA 7<br><br>EUS-B: 1x FNA 7 | yes | Intranodal metastases |
| M, 57y | Peripheral, LUL | Adenocarcinoma | cT2aN2M0 | 4L, 5,6 | no (5,6) and yes (4L) | EBUS: 2x TBNA 4L, no TBNA 5 and 6<br><br>EUS-B: 4x FNA 4L, no FNA 5 and 6 | yes and no | Micrometastasis 4L; Extranodal 5,6<br><br>MS also FN result, LND revealed nodal metastasis |
| F, 62y | Centrally, LUL | SqCC | cT2bN0M0 | 5 | no | ÈBUS: No sampling FN node<br><br>EUS-B: No sampling FN node | no | Intranodal metastases |
| M, 77y | Centrally, LUL | SqCC | cT3N2M0 | 6 | yes | ÈBUS No sampling FN node | no | Unknown. No surgical verification, treated with chemoradiotherapy, suggestion of growth of LN after |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | EUS-B No sampling FN node | | treatment. |
| M, 69y | Peripheral, RLL | SqCC | cT3N2M0 | 3A, Anterior of VCS | yes | ÈBUS No sampling FN node<br><br>EUS-B No sampling FN node | no | Intranodal metastases |
| M, 64 | Centrally, LUL | SqCC | cT2bN2M0 | 5 | yes | ÈBUS No sampling FN node<br><br>EUS-B No sampling FN node | no | After induction chemotherapy CT scan showed a reduction in LN size, subsequently a lobectomy with LND was performed: pT4N0. Based on imaging prior to surgery the endosonography results were considered to be FN. |

Legend Supplement 3.

Supplement 3 shows additional information of patients in whom endosonography results were false negative.

Abbreviations:

MS: Mediastinoscopy; LND Lymph Node Dissection; EBUS: endobronchial ultrasound; EUS-B: endoscopic ultrasound using the EBUS scope; RLL: Right Lower Lobe, RUL: Right Upper Lobe; LLL: Left Lower Lobe; LUL: Left Upper Lobe; TBNA: Transbronchial Needle Aspiration; FNA: Fine Needle Aspiration; FN: False Negative;